

IN THE CLAIMS:

1. (Currently amended) An antibody microarray screening method including the steps of depositing a plurality of spots of antibodies selected from the group consisting of purified immunoglobulins and fluids that are unprocessed for immunoglobulin isolation on predetermined positions on a said substrate; comprising: a substrate; monoclonal and polyclonal antibodies that are purified immunoglobulins, wherein said antibodies are spotted hybridizing the plurality of spots of antibodies with a plurality of labeled target proteins; quantifying the label signal strength produced at each spot; quantifying levels of target protein captured at each spot on the basis of label signal strength; and comparing levels of target protein among the plurality of samples of labeled target proteins.

~~-and fluids unprocessed for immunoglobulin isolation, wherein said unprocessed fluids with higher amounts of proteins than said purified immunoglobulin proteins to compensate non-immunoglobulin proteins in said unprocessed fluids are spotted on said predetermined positions on said substrate.~~

2. (Currently amended) The antibody microarray screening method according to claim 1, wherein said the step of depositing a plurality of spots of antibodies is further defined as depositing a plurality of spots of antibodies specific for proteins selected from the group consisting of drug-metabolizing enzymes and proteins functionally related with said drug-metabolizing enzymes.

3. (Currently amended) The antibody microarray screening method according to claim 2, wherein the step of depositing a plurality of spots of antibodies specific for proteins selected from the group consisting of drug-metabolizing enzymes and proteins functionally related with said drug-metabolizing enzymes is further defined as depositing a plurality of spots of

antibodies specific for ~~said drug-metabolizing enzyme is cytochromes P450.~~

4. (Currently amended) The antibody microarray screening method according to claim 2, wherein said the step of depositing a plurality of spots of antibodies specific for proteins functionally related with said drug-metabolizing enzymes is further defined as depositing a plurality of spots of antibodies specific for ~~said proteins functionally related with said~~ at least one drug-metabolizing enzyme are selected from the group consisting of mitochondrial proteins, apoptosis-related proteins, anti-oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins.

5. (Currently amended) The antibody microarray screening method according to claim 1, wherein the step of depositing a plurality of spots of antibodies on predetermined positions on a substrate is further defined as depositing a plurality of spots of antibodies on predetermined positions on a hydrogel (polyarylamide-based) coating.

6. (Currently amended) The antibody microarray screening method according to claim 1, further including the step of ~~and~~ determining optimal spotting concentrations of said purified immunoglobulins and the optimal dilution factors of said fluids that are unprocessed for immunoglobulin isolation. ~~IgG. by further comprising steps of (a) hybridizing the slides with labeled secondary immunoglobulins, (b) scanning and quantitating signal strength of each spot, and (c) selecting optimal concentrations of IgG using %visible IgG spots.~~

7. (Currently amended) The antibody microarray screening method according to claim 1, wherein the step of depositing a plurality of spots of antibodies selected from the group consisting of purified immunoglobulins and fluids that are unprocessed for immunoglobulin isolation is further defined as

depositing a plurality of spots of antibodies selected from the group consisting of purified immunoglobulins and fluids that are selected from the group consisting of ascites fluids, hybridoma culture medium, and anti-sera.

8. - 11 (Cancelled)

12. (Withdrawn) An antibody microarray screen comprising: a substrate; monoclonal antibodies as purified immunoglobulins, wherein said antibodies are spotted on predetermined positions on said substrate; ascites fluid spotted on said substrate; and hybridoma culture media spotted on said substrate, wherein said ascites fluid and hybridoma culture media with higher amounts of proteins than said purified immunoglobulin proteins to compensate non-immunoglobulin proteins in said unprocessed fluids are spotted on predetermined positions on said substrate.

13. (Withdrawn) The antibody microarray screen according to claim 12, wherein said monoclonal antibodies detect proteins selected from the group consisting of drug-metabolizing enzymes, cytochromes P450, and oxidative stress proteins.

14. (Withdrawn) The antibody microarray screen according to claim 12, wherein said substrate includes a hydrogel (polyarylamide) coating.

15. (Withdrawn) The antibody microarray screen according to claim 12 and determining optimal spotting concentrations of IgG by further comprising steps of (a) hybridizing the slides with labeled secondary immunoglobulins, (b) scanning and quantitating signal strength of each spot, and (c) selecting optimal concentrations of IgG using %visible IgG spots.

16. (Withdrawn) A method of manufacturing an antibody microarray comprising the step of spotting more than a single concentration of antibodies on a microarray substrate to increase the number of up-regulated protein detection.

17. (Withdrawn) The method according to claim 16, wherein the antibody concentration is more than 5 µg/ml IgG.

18. (Withdrawn) An internal control molecule for use in an antibody microarray comprising a protein, wherein said protein is unexpressed in the array sample for normalization of focused (non-global) array data.

19. (Withdrawn) The internal control molecule according to claim 18, wherein said protein is selected from the group consisting of a Flag protein and a non-mammalian protein.

20. (Withdrawn) The internal control molecule according to claim 18, wherein the internal control molecule is used to compare the expression ratio of house-keeping proteins to select housekeeping genes by determining any difference between the control and experimental samples.

21. (Withdrawn) A method of determining optimal spotting concentrations of IgG comprising the steps of: (a) spotting increasing concentrations of IgG on microarray slides; (b) hybridizing the slides with secondary IgG with a detectable signal; and (c) scanning and quantitating signal strength of each spot and selecting optimal concentrations of IgG.

22. (Withdrawn) A method to increase a detectable signal with microarray analysis comprising the steps of using an intensive molecular signal, wherein the intensive molecular signal is produced by conjugation of a dye and a reporter molecule to a protein whereby interference of IgG binding to a protein is

created.

23. (Withdrawn) The method according to claim 22, wherein the intensive molecular signal is produced by conjugation of a dye and a reporter molecule to a protein to the extent that interference of Coomassie blue stain binding to the protein is created.

24. (Withdrawn) The method according to claim 22, wherein the intensive molecular signal is used for antibody microarrays.

25. (Withdrawn) The method according to claim 22, wherein the intensive molecular signal is used for protein microarrays.

26. (Withdrawn) A method to increase a detectable signal with microarray analysis comprising the steps of: conjugating of a dye and a reporter molecule to a protein; and creating interference of an IgG molecule binding to the protein.

27. (Withdrawn) A method of producing antibody microarrays comprising the steps of spotting antibodies for Phase I and II drug metabolizing enzymes and proteins functionally related with the drug-metabolizing enzymes on a microarray substrate.

28. (Withdrawn) The method according to claim 27, wherein the proteins functionally related with drug-metabolizing enzyme are selected from the group consisting of mitochondrial proteins, apoptosis-related proteins, anti-oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins.

29. (Withdrawn) The method according to claim 27, wherein the targeted drug-metabolizing enzyme antibody microarray includes an internal control to be

used for data normalization.

30. (Withdrawn) The method according to claim 29, wherein the internal control is a Flag protein.

31 (New). The antibody microarray screening method according to claim 6, wherein the step of determining optimal spotting concentrations of said purified immunoglobulins and said fluids that are unprocessed for immunoglobulin isolation further includes the steps of depositing, at a first set of locations, a plurality of spots of at least one purified immunoglobulin at a plurality of concentrations; depositing, at a second set of locations, a plurality of spots of at least one fluid that is unprocessed for immunoglobulin isolation at a plurality of dilution factors; hybridizing the spots with labeled secondary immunoglobulins; scanning and quantifying the signal strength of label at each spot; selecting an optimal concentration of the at least one purified immunoglobulin on the basis of the production of an optimal label signal strength; and selecting an optimal dilution factor of the at least one fluid that is unprocessed for immunoglobulin isolation by selecting a dilution factor that yields a label signal strength similar to that produced by the optimal concentration of the at least one purified immunoglobulin.

32. (New) The antibody microarray screening method according to claim 31 wherein the step of scanning and quantifying the signal strength of label at each spot is further defined as scanning and determining the percentage of visible spots in the scanned image.

33. (New) The antibody microarray screening method according to claim 1 wherein the step of depositing a plurality of antibody spots on predetermined positions on a substrate is further defined as spotting fluids that are unprocessed for immunoglobulin isolation at higher amounts of protein than purified

immunoglobulins, thereby compensating for the lower concentrations of antigen specific immunoglobulins in said fluids that are unprocessed for immunoglobulin isolation.

34. (New). The antibody microarray screening method according to claim 32 wherein the step of depositing a plurality of antibody spots on predetermined positions on a substrate is further defined as spotting fluids that are unprocessed for immunoglobulin isolation at a 10 – 1000 fold dilution and spotting purified immunoglobulins at a protein concentration of 5-500 µg/ml.